Contaminants of Emerging Concern in Urban Wastewater

Joint NORMAN and Water Europe Position Paper

This Position Paper, supported by the NORMAN network and Water Europe, provides a series of recommendations to the European Commission for consideration as part of their review of the Urban Waste Water Treatment Directive and the fitness check of the EU Water Framework Directive.

With various existing EU Directives undergoing evaluation and new initiatives under development (e.g. Water Reuse Regulations), the EU water policy is undergoing major changes to better meet new transboundary challenges, including climate change, urbanisation, water scarcity and emerging sources of water pollution.

In support of this policy review process, various EU projects (such as the NEREUS COST Action, FRAME, ANSWER, PROMOTE, SOLUTIONS etc.) have been funded to tackle the problems of contaminants of emerging concern (CECs) in urban wastewater and the definition of quality criteria (including chemical and microbiological parameters) for water reuse.

In addition to that, several investigative campaigns are taking place in Europe at the national and international level (e.g. Danube river basin) and as part of the NORMAN Joint Programme of Activities to identify priority CECs in wastewater effluents.

Significant progress has also been made in the development of advanced analytical tools – such as high-resolution mass spectrometry (HRMS) – for the determination and identification of CECs (and their degradation products) as well as the use of ecotoxicological tests to enable the impacts of pollutant cocktails to be considered within regulatory frameworks.

In light of the conclusions and data generated by all these initiatives, a workshop on *Prioritisation of Emerging Contaminants in Urban Wastewater* was organised (6 March 2019, Palaiseau, Paris) by the NORMAN network (www.norman-network.net) in the framework of the AQUAlity ETN project and with the support of Water Europe (www.watereurope.eu), with the following objectives:

- Critically analyse and consolidate the results of relevant EU and national projects which have recently been funded in support of the on-going policy evaluation process (Urban Waste Water Treatment Directive, Water Framework Directive, etc.)
- Provide a platform for leading experts to discuss key issues including: Is it possible to define a common list of European “priority” CECs in urban wastewater? What should be the priority target for innovative treatment technologies?
- Is it necessary to set new emission limit values (ELV) for specific priority (groups of) contaminants, or effects?

As a result of the workshop, the participants agreed on the following conclusions and recommendations.

RECOMMENDATIONS

1. *Introduce specific measures to address Contaminants of Emerging Concern (CECs) in the Urban Waste Water Treatment Directive (UWWTD)*

The current version of the UWWTD is not 'in phase' with the substantial evidence base that the occurrence of CECs in the environment is an issue of concern for exposed ecosystems and human health. CECs are released into the environment as a result of anthropogenic activities, with a trend of increasing load and types of pollutants due to population growth and the escalating introduction of new chemicals to the market.

Not all CECs are persistent, but due to their continuous use and discharge into the environment, many of them are regularly found in the environment and can accumulate in food webs. Moreover, although many chemicals are only used in small quantities which may be considered harmless, there is increasing concern about mixture – or cocktail – effects arising from the multitude of chemicals present in our environment.

Improved pollution prevention measures should be promoted as a priority. Discharges from wastewater treatment plants (WWTPs) are major points of release of CECs into the environment and their mitigation has an important role in pollution prevention.

> *The updated UWWTD should introduce proactive measures to reduce the emissions of CECs discharged in WWTP effluents and stormwater runoff.*

Whilst reduction-at-source and substitution measures can play a role in reducing emissions, these approaches are not feasible in all circumstances and hence, alone, will not be sufficient to achieve the goal of protecting the environment against adverse effects of wastewater effluents and stormwater discharges from urban and industrial activities. It is also stressed that urban WWTPs collect the emissions from upstream sources, including in some cases industrial sites, and therefore source-related measures should be more systematically applied in line with the "polluter pays" principle.

> *The updated UWWTD should apply monitoring schemes able to discriminate between the normal WWTP effluent patterns and specific emissions (e.g. from specific industrial activities) requiring source-related measures according to the "polluter pays" principle.*

2. *Provide incentives to develop and implement innovative treatment technologies to focus on changes in the pollutant emissions/behaviour associated with the priority challenges of climate change and population growth*

The priority target pollutants for treatment may depend on the country (e.g. industrial activities, land use, climate and socio-economic status) and, more specifically, on the receiving water body (e.g. inherent bio-physico-chemical characteristics) and river basin hydrology and geology. However, as a general requirement, authorities – at both national and European level – should provide incentives for investment in innovative treatment technologies able to tackle challenges related to 1) the effects of climate change (increasing water scarcity, reduced dilution of WW discharges in surface water, higher variability in precipitation), 2) demographic development (increasing pollutant loads) and 3) the implementation of the principles of a circular economy and the reuse of wastewater as a resource (water, dissolved nutrients, nutrients in the sludge, energy).

To make the treatment options 'future-proof', the following considerations should be taken into account:

- treatment technologies should cover a broad spectrum of CECs with different properties (e.g. hydrophobic/hydrophilic). This is necessary to create enough long-term flexibility for changing conditions due to demographic development and climate change;
- treatment efficiency should be adaptable to concentrations in the wastewater and should be targeted towards a specified effluent water quality;
- treatment technology should be efficient in energy and chemicals demand and have a low environmental impact;
- treatment of CECs should not hinder the development of circular economy principles of recovering water and valuable resources from the treated wastewater.

Powdered Activated Carbon (PAC) treatment and ozonation are currently considered as consolidated advanced treatment technologies. However, both technologies have a relatively high energy consumption, and the use of activated carbon creates a residual spent carbon. Also, the production of activated carbon has a high environmental impact.

A major limitation at present is insufficient implementation of the Best Available Techniques (BATs) concept at European level:

- not all discharges are yet connected to sewer systems;
- not all sewage is yet treated;
- tertiary (or even secondary) treatment is not yet performed properly everywhere;
- many problems are still affecting the performance of smaller WWTPs;
- BAT might not even be sufficient to reduce all risks.

These problems appear to be due to insufficient pressures being placed on WWTP operators or large industrial installations to reduce emissions. There also seems to be a lack of:

- political stringency at the national or local level;

- sufficiently demanding European regulations to enforce, and;
- inspectors to monitor levels of compliance.

Therefore, it is necessary to use all available means to improve communication, cooperation and involvement of all stakeholders (policy-makers, WWTP operators, water agencies and industry) with a view to increasing and facilitating the transfer of knowledge and technology from R&D to practice.

> *Governments should financially support (e.g. via tax exemption) WWTPs that introduce new treatment technologies.*

3. Define criteria for the selection of urban WWTP facilities to be upgraded

There is widespread agreement among experts that at least a part of the existing urban WWTPs in Europe should be upgraded.

Respective WWTPs should be selected according to one or more of the following criteria:
- large WWTPs (to be specified[1]) (to significantly reduce the load)
- WWTPs with a high fraction of wastewater with respect to the river size (to be specified at river basin level [1])[2] (to protect the aquatic ecosystems)
- WWTPs that influence drinking water resources
- WWTPs that influence valuable ecosystems (e.g. Natura 2000) [2].

> *It is important to define overall abatement objectives at EU level for CECs released from WWTPs and to guide managers and decision-makers towards the minimum improvements needed.*

4. Define measurable objectives for the reduction of CECs in urban WWTP effluents

The final target is to achieve an acceptable level of risk associated with the occurrence of CECs in the receiving water body (recognising that this varies from site to site). As a general requirement, WWTP operators should install appropriate abatement technologies able to clean wastewater to a level defined as posing an acceptable level of environmental risk.

> *In more operational terms, a first general reduction objective, associated with the performance of advanced treatment technologies, should be defined. The reduction target could be expressed as x% abatement of CECs load over the whole treatment, in line with the model applied in the Swiss Water Protection Act.*

[1] In the Swiss Water Protection Act: Large WWTPs are installations > 80,000 inhabitants
[2] The criteria and thresholds should be defined at river basin level

The target for the reduction of CECs in WWTP effluents applied in the new Swiss Water Protection Act is an abatement by 80%, to be evaluated as average abatement of selected indicator substances over the whole treatment. The Swiss Water Protection Act has been in force since January 2016 with an implementation time frame up to 2040 ([3, 4]).

The Swiss model could be adopted at the European scale as part of the updated UWWTD. However, it should be noted that a reduction of 80% is defined as a general objective and is not meant to take into account local risks. It may not be sufficient to reduce the impact of specific CECs that represent a major risk at the respective sites. The abatement might therefore need to be adjusted to a higher target to account for specific local conditions (sensitive areas, smaller water bodies, etc.).

> *To take into account local risks, a complementary risk-based approach should be applied, where specific pollutants (toxicity drivers) would be identified and higher emission reduction objectives would be defined at local level to ensure that Emission Limit Values (ELVs) for relevant pollutants or groups of pollutants are complied with (see Recommendations 6 and 7).*

5. Define performance indicators to ensure the effectiveness of the abatement processes in the upgraded WWTPs

A prerequisite to ensure that the target objectives for abatement of CECs are met is the definition of a set of performance indicators which facilitate the systematic assessment of the performance of a wastewater abatement process.

> *The updated UWWTD should define a common list of criteria to be used at EU level for selection of performance indicators necessary to evaluate the effectiveness of the abatement process and the achievement of the target CECs reduction objectives in the upgraded WWTPs.*

Based on current experience, the compounds to be selected as performance indicators should be compounds which:

- are continuously discharged and regularly found in the influent of WWTPs,
- occur in most WWTP effluents at measurable concentrations (preferably, the ratio between the measured environmental concentration and the method detection limit should exceed 10[3]),
- can be easily and routinely measured by as few as possible (optimally one) analytical methods,
- broadly cover the range of physico-chemical properties and biodegradability affecting their removal by the various treatment processes,
- broadly represent the range of treatability features; from "biodegradable during conventional activated sludge treatment or biofiltration", to "not degradable during conventional activated

[3] As an alternative option, in the Swiss Water Protection Act, the Limit of Quantification (LOQ) for the analysis of the indicator substances in the effluent of the WWTP must be 10 times smaller than the concentration in the influent of the WWTP, so that it is possible to quantify an abatement during wastewater treatment of at least 90%.

sludge treatment or biofiltration, but amenable to chemical oxidation or sorption to AC", and "not degradable during conventional activated sludge treatment or biofiltration, and not amenable to chemical oxidation or sorption to AC",
- undergo a similar degree of abatement in advanced treatment technologies (e.g. ozonation or sorption to AC).

> *The above-listed criteria should serve as a basis for the definition of a comprehensive list of compounds frequently found in urban wastewater in Europe, which could be grouped in the following three categories:*
> - *"readily biodegradable",*
> - *"not biodegradable but amenable to chemical oxidation or sorption to AC",*
> - *"not biodegradable and not amenable to oxidation or sorption to AC".*

At the national level, WWTP operators could select, from a common list, a minimum number of performance indicators representative and fit for purpose for the WWTPs in their country (10-12 compounds, with 3-4 compounds for each of the above-mentioned categories).

Two examples of lists of WWTP performance indicators are provided in the Annex: the list applied in the Swiss Water Protection Act (Annex 1) and the list developed within the NEREUS COST Action (Annex 2).

Transparency is needed around the selection of chemicals, i.e. stakeholders, including scientists, should know the criteria for choices.

6. Assess the overall mixture toxicity of effluents with a battery of bioassays and define a list of local / site-specific pollutants in order to ensure the protection of water bodies

Another more stringent requirement in addition to the list of performance indicators mentioned above is to use a battery of bioassays (*in vitro* and *in vivo*) and associated Effect-based Trigger Values (EBTs)[4] [5] as a "safety net" at the outlet of the WWTP.

In order to ensure that the "safety net" goal is met, it is strongly suggested [2] that the battery of effect-based monitoring tools should include both *in vivo* and *in vitro* assays. Using *in vitro* assays, only a limited fraction of modes of action can be detected. *In vivo* tests are needed to address apical effects that cannot be covered with *in vitro* tests.

> *The updated UWWTD should require the application of a battery of bioassays (in vitro and in vivo) and associated EBTs as a way to assess the risks associated with effluent mixtures (containing a*

[4] The activity report of the EBM Working Group (EC DG-ENV/WG-Chemicals) proposed a tiered approach to determine EBTs for a given endpoint, depending on available data.

range of both known and unknown pollutants) released from WWTPs and thus to ensure the protection of the water body from residual chemical contaminants.

If one or more EBTs are exceeded the WWTP operator should take actions to identify both the pollutants (toxicity drivers) responsible for the observed effects and their sources, and adopt measures in line with the 'polluter pays' principle.

It is important to note that the toxicity drivers may be different from the compounds identified as *WWTP performance-related indicators* and that they depend on the type of input received by the WWTP (most WWTPs receive not only municipal wastewater but also wastewater from industry, agricultural areas, etc.).

When the cause/source of the observed effect is known, water managers can apply upgrading measures without the need for chemical analysis for identification of the specific toxic driver(s). In this case, it is sufficient to take measures at the source and measure the improvement by bioassays.

An example of a list of endpoints (and proposed *in vitro* bioassays) and a graduated approach with actions to be taken by WWTP operators in case of exceedance of EBTs was developed within the NEREUS COST Action and is reported in Annex 2 – Table 3 (list of endpoints) and Table 4 (proposed actions). For each endpoint, it is possible to identify classes of chemicals which may cause the observed effect.

When it is not possible for water managers to identify the pollution source(s), identification of the relevant toxicity drivers can be done using analytical screening techniques (wide-scope target screening and suspect screening) with quantification of target chemicals and component-based risk assessment of the mixtures.

Explaining the observed activity detected by the applied bioassays can be done (using mass balances/ "iceberg modelling") by calculation of Toxic Units (TU) for each of the quantified pollutants (separately for algae, invertebrates and fish) and Bioanalytical Equivalent concentration (BEQ) for endocrine disruptors depending on the bioassay. This should be followed by a comparison of the estimated $\sum$TU or BEQ from the component-based assessment with the TU and BEQ derived from the bioassay testing. If EBTs are exceeded and the component-based assessment cannot explain the activity detected in the bioassay, an Effect-Directed Analysis (EDA) protocol should be performed in order to identify the risk drivers and find optimum ways to solve the problem at source ([6, 7], [[8, 9]).

7. Define a list of WWTP-related pollutants as candidates for the list of river basin-specific pollutants

In a similar way to the approach mentioned above for the identification of local risk drivers, a list of candidate river basin-specific pollutants (RBSPs) could be identified in order to address potential risks of WWTP-related pollutants on a larger scale, i.e. not only at the individual WWTP level. For this purpose, it would be advisable to introduce a procedure (e.g. Watch List mechanism) to collect evidence of the widespread presence and potential risks of these pollutants from wastewater effluents.

To that purpose, Emission Limit Values (ELV) should be set based on local conditions, following a harmonised EU protocol.

ELVs could be derived from EQS values for each individual compound, taking into account the local dilution factor of the WWTP, as follows:

- *ELV = EQS x dilution factor*

where the dilution factor varies between 2 and 10 for the majority of sites and depends on the size of the receiving water body and the size of the WWTP.

Provided that EQSs are defined at EU level for all the compounds on the list (including performance indicators mentioned above), it will be possible to derive ELVs for each of these compounds.

When it is necessary to take into account multiple sources (e.g. upstream WWTPs) for specific individual compounds in a given river basin, it is suggested that WWTP operators should apply an additional safety factor (e.g. 2 – 5) for calculation of the ELV (i.e. ELV = (EQS x dilution factor)/safety factor) for all WWTPs within the river basin. This safety factor should be proposed and justified by the responsible river basin authority.

> *Several compounds are already identified as suspect contaminants frequently found in urban WW effluents and potentially causing risks to the ecosystems and human health. A list of candidate pollutants reflecting pressures from WWTPs at river basin level should, therefore, be defined and regularly monitored to decide on their final status as RBSPs. Exceedance of ELVs (equivalent to exceedance of EQS in surface water) should trigger identification of a compound as a candidate RBSP.*

8. Ensure regular revision of the lists of WWTP-related CECs to be monitored and controlled by WWTP operators and environmental authorities

Based on experiences from the regular application of bioassays, improvements in analytical methods or long-term monitoring, the lists of compounds that are suspected to cause local or river basin-wide risks, or that best represent the performance of abatement of new treatment technologies, might usefully be revised.

> *The update of the UWWTD should integrate a mechanism for regular revision/update of the above-mentioned lists of CECs every five years.*

9. Use various existing tools to monitor the performance of the treatment process

While chemical analysis is compulsory to monitor the performance of the treatment process, effect-based tools (battery of bioassays) could also be used to monitor the potential effects of chemicals in outfalls/receiving waters. The choice of the bioassays should be adapted according to the final use of the water (reuse for irrigation purposes in agriculture, drinking water production or other uses) or its emission into receiving water bodies (e.g. emission into a river or the sea). Although there is as yet no common agreement at an EU level on a list of monitoring criteria to select the most suitable bioassays, these tools are included in the legislation of some Member States in specific contexts. They should therefore certainly be considered in the upcoming update of the UWWTD.

> *To monitor the impact of wastewater, a combination of chemical and biological analysis should be used.*

On a day-to-day basis, the performance of wastewater treatment is currently monitored online by sensors for general physico-chemical parameters such as pH, temperature, turbidity, Oxidation-Reduction Potential (ORP), dissolved oxygen (DO), UV absorption to control ozone or PAC dosage, etc. Specific chemical sensors for CECs would be useful for effluent monitoring or in-process monitoring, to tell operators whether the treatment plant is working efficiently and whether advanced treatment is needed at a certain time. This decision support would be very beneficial in saving energy and ensuring compliance. However, sensing technologies for most emerging contaminants are not available or not yet robust enough for routine application. Sensors and biosensors should be developed especially for parameters that are indicative of treatment performance and / or occurrence in the influent.

> *Improved sensing technologies are needed to monitor everyday treatment performance. Continued and greater investment in sensing and biosensing research is needed.*

10. Emissions from stormwater runoff and combined sewer overflows

Next to WWTPs, stormwater runoff and combined sewer overflows (CSOs) are major sources for CECs (and pathogens) emissions to surface waters. Moreover, the pollutants enter the surface water untreated. Technical measures in the design of the sewer system are required to reduce emissions through this pathway to a minimum. Because of the effects of climate change (increasing intensity and frequency of rainfall) this aspect is becoming increasingly important.

> *The update of the UWWTD should include regulations for the maximum allowance of CSO events in terms of volume and frequency.*

Acknowledgements

The authors would like to acknowledge Paola Calza (University of Torino, Italy), coordinator of the ETN AQUAlity project (funded under the European Union's Horizon 2020 research and innovation programme - Marie Skłodowska-Curie grant agreement N. 765860), which enabled the organisation of the workshop "Prioritisation of Emerging Contaminants in Urban Wastewater" (6 March 2019, Ecole Polytechnique, Palaiseau, Paris, France).

Workshop organisers

Valeria Dulio (*corresponding author*: valeria.dulio@ineris.fr) and Azziz Assoumani - INERIS, France

Speakers and contributors to the position paper

Name		Institute	Country
Valeria	Dulio*	INERIS	France
Jan	Hofman	Water Europe and University of Bath	UK
Peter	von der Ohe	UBA	Germany
Werner	Brack	UFZ	Germany
Christa	McArdell	Eawag	Switzerland
Jaroslav	Slobodnik	Environmental Institute	Slovakia
Sara	Valsecchi	CNR-IRSA	Italy
Fiona	Regan	Dublin City University	Ireland
Jenny	Lawler	Dublin City University	Ireland
Blanaid	White	Dublin City University	Ireland
Selim	Aït-Aïssa	INERIS	France
Thorsten	Reemtsma	UFZ	Germany
Hélène	Budzinski	University of Bordeaux	France
Azziz	Assoumani	INERIS	France
Pierre-françois	Staub	French Agency for Biodiversity (AFB)	France

Workshop participants list

Name		Institute	Country
Laura	Achene	Istituto Superiore di Sanità	Italy
Harsh	Agarwal	Ecole Polytechnique	France
Anandita	Agarwal	Ecole Polytechnique	France
Selim	Aït-Aïssa	INERIS	France
Maria de Fátima	Alpendurada	IAREN - Instituto da Água da Região Norte	Portugal
İlknur	Altın	Karadeniz Technical University	Turkey
Chukwuka Bethel	Anucha	Karadeniz Technical University	Turkey
Azziz	Assoumani	INERIS	France
Emin	Bacaksız	Karadeniz Technical University	Turkey
Masho Hilawie	Belay	University of Piemonte Orientale	Italy
Faten	Belhaj	Veolia	France
Stéphanie	Bémelmans	ISSeP	Belgium
Staffan	Bergström	Biotage Sweden AB	Sweden
Ilaria	Berruti	PSA-CIEMAT	Spain
Alessandra	Bianco Prevot	University of Torino	Italy
Rita	Binetti	SMAT	Italy
Fabrício Eduardo	Bortot Coelho	University of Torino	Italy
Stéphane	Bouchonnet	Ecole Polytechnique	France
Sophie	Bourcier	Ecole Polytechnique	France
Werner	Brack	UFZ	Germany
François	Brion	INERIS	France
Hélène	Budzinski	University of Bordeaux	France

Paola	Calza	University of Torino	Italy
Francesca	Cappelli	IRSA-CNR	Italy
Clemence	Chardon	INERIS	France
Dennis	Deemter	PSA-CIEMAT	Spain
Donata	Dubber	Environmental Protection Agency	Ireland
Valeria	Dulio	INERIS	France
Onno	Epema	RWS	The Netherlands
Esra	Eray	LIQTECH International	Denmark
Mar	Esperanza	SUEZ	France
Rodolphe	Gaucher	INERIS	France
Nuno	Gonçalves	University of Torino	Italy
Olivier	Gras	French Ministry of Environment	France
Lauriane	Greaud	INERIS	France
Teysir	Guefrachi	Ecole Polytechnique	France
Imogen	Hands	Dublin City University	Ireland
Jan	Hofman	University of Bath and Water Europe	UK
Barrie	Howe	Environment Agency England	UK
Katarzyna	Janowska	Aalborg University	Denmark
Cristina	Jiménez Holgado	University of Ioannina	Greece
Cécile	Kech	ISSeP	Belgium
Philippe	Lacroix	French Ministry of Environment	France
Jenny	Lawler	Dublin City University	Ireland
Mauricio	Lelis Viotti Cavalin	École Polytechnique	France
Katharina	Lenz	Environment Agency	Austria

Francois	Lestremau	INERIS	France
Julio	Llorca	Labaqua	Spain
Sixto	Malato	PSA-CIEMAT	Spain
Lucile	Marsollier	French Ministry of Environment	France
Lara	Matragi	Ecole Polytechnique	France
Christa	McArdell	Eawag	Switzerland
Regina	McGinn	Environmental Protection Agency	Ireland
Regis	Moilleron	LEESU	France
Susanna	Murtas	Istituto Superiore di Sanità	Italy
Isabel	Oller	PSA-CIEMAT	Spain
Davide	Palma	CNRS, University Clermont Auvergne	France
Claudia	Paijens	LEESU	France
Dimitra	Papagiannaki	SMAT	Italy
Alice	Pavanello	Universitat Politecnica de Valencia	Spain
Maria Inmaculada	Polo López	PSA-CIEMAT	Spain
Fiona	Regan	Dublin City University	Ireland
Claire	Richard	CNRS, Institut de Chimie de Clermont-Ferrand	France
Elisa	Robotti	University of Piemonte Orientale	Italy
Erwin	Roex	Deltares	The Netherlands
Iván	Sciscenko	Universitat Politecnica de Valencia	Spain
Jaroslav	Slobodnik	Environmental Institute	Slovakia
Pierre-françois	Staub	AFB	France
Gunnar	Thorsén	IVL Svenska Miljöinstitutet	Sweden

Giorgio	Tomasi	University of Copenhagen	Denmark
Sara	Valsecchi	IRSA-CNR	Italy
Zsuzsanna	Varga	Ecole Polytechnique	France
Blánaid	White	Dublin City University	Ireland
Thorsten	Reemtsma	UFZ	Germany
Peter	von der Ohe	UBA	Germany

References

1. Link, M., et al., *Comparison of dilution factors for German wastewater treatment plant effluents in receiving streams to the fixed dilution factor from chemical risk assessment.* Science of The Total Environment, 2017. **598**: p. 805-813.
2. Coppens, L.J., et al., *Towards spatially smart abatement of human pharmaceuticals in surface waters: Defining impact of sewage treatment plants on susceptible functions.* Water Res, 2015. **81**: p. 356-65.
3. Eggen, R.I., et al., *Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants.* Environ Sci Technol, 2014. **48**(14): p. 7683-9.
4. Bourgin, M., et al., *Evaluation of a full-scale wastewater treatment plant upgraded with ozonation and biological post-treatments: Abatement of micropollutants, formation of transformation products and oxidation by-products.* Water Res, 2018. **129**: p. 486-498.
5. Escher, B.I., et al., *Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive.* Sci Total Environ, 2018. **628-629**: p. 748-765.
6. Brack, W., et al., *Effect-directed analysis supporting monitoring of aquatic environments — An in-depth overview.* Science of The Total Environment, 2016. **544**: p. 1073-1118.
7. Brack, W., et al., *Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality.* Environmental Sciences Europe, 2019. **31**(1): p. 10.
8. Neale, P.A., et al., *Linking in vitro effects and detected organic micropollutants in surface water using mixture-toxicity modeling.* Environ Sci Technol, 2015. **49**.
9. König, M., et al., *Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis.* Environ Pollut, 2017. **220**.
10. NEREUS project. *New and Emerging challenges and opportunities in wastewater REUSe - ES1403* http://www.nereus-cost.eu/. [cited 2017.
11. Alygizakis, N.A., et al., *Characterization of wastewater effluents in the Danube River Basin with chemical screening, in vitro bioassays and antibiotic resistant genes analysis.* Environment International, 2019. **127**: p. 420-429.

Annex 1- List of performance indicators used in Switzerland

In Switzerland, a list of 12 indicator substances (see table below) was chosen to evaluate the effectiveness of wastewater treatment in WWTPs which have implemented advanced treatment with either ozone or activated carbon. To ensure the efficiency of the upgraded wastewater treatment, these 12 indicator substances must be abated on average by 80% over the whole plant. The compounds were specifically chosen as non-easily biodegradable substances and are therefore normally not well removed during conventional biological wastewater treatments ([3, 4]).

Table 1 – List of 12 performance indicator implemented in Switzerland to ensure the efficiency of the upgraded wastewater treatment plants

Substance	Class	Abatement during ozone or PAC treatment
Amisulpride	Pharmaceutical (antipsychotic)	Very good (>80%)
Carbamazepine	Pharmaceutical (antiepileptic)	Very good
Citalopram	Pharmaceutical (antidepressant)	Very good
Clarithromycin	Pharmaceutical (macrolide antibacterial)	Very good
Diclofenac	Pharmaceutical (anti-inflammatory / antirheumatic)	Very good
Hydrochlorothiazide	Pharmaceutical (diuretic)	Very good
Metoprolol	Pharmaceutical (beta blocking agent)	Very good
Venlafaxine	Pharmaceutical (antidepressant)	Very good
Benzotriazole	Corrosion inhibitor	Good (50-80%)
Methylbenzotriazole	Corrosion inhibitor	Good
Candesartan	Pharmaceutical (antihypertensive agent, angiotensin II antagonist)	Good
Irbesartan	Pharmaceutical (antihypertensive agent, angiotensin II antagonist)	Good

Annex 2- NEREUS COST Action proposal: parameters to be included in WWTP effluents monitoring programmes

A minimum list of indicator substances to assess the performance of the wastewater abatement process and ensure that the target objectives for abatement of pollution are achieved with appropriate surrogate parameters in treated wastewater and frequency of their monitoring should be established by the Commission. The Commission should be responsible for defining threshold values for the indicator chemicals at the EU level, whereas Member States (MS) could define more stringent threshold values at a national, river basin or other level addressing regional or local conditions.

It is recommended that an initial list should contain approximately 10 substances and it should indicate the possible methods of analysis not entailing excessive costs for each substance. A mechanism for regular revision/update of the list of CECs every five years should be proposed by the Commission. A list of initial CECs is provided in Table 1. The chemicals were proposed based on the experience and European data available to the NEREUS experts [10] with reference to Australian and Californian water reuse legislation (NRMMC & EPHC & NHMRC, 2008; Drewes, et al., 2013). For the general criteria used for the selection of the substances, see the footnote of Table 1 and text.

Table 1 - Proposed list of initial CECs to be included in WWTP effluent monitoring programmes

Indicator chemical	ELV= PNEC[5]*dilution factor (ng/L)	Frequency[4]	References - analytical method
Biodegradable[1]			
Benzotriazole	tba	Every 6 months	Loos et al., 2013
Diclofenac	tba	Every 6 months	Loos et al., 2013
Gabapentin	tba	Every 6 months	Kasprzyk-Horderna et al., 2008
Trimethoprim	tba	Every 6 months	Kostich et al., 2014
Sulfamethoxazole	tba	Every 6 months	
Valsartanic acid	tba	Every 6 months	Schultz et al., 2010
Oxypurinol	tba	Every 6 months	Funke et al., 2015
Not biodegradable, but oxidizable[2]			
Acesulfam	tba	Every 6 months	Loos et al., 2013
Carbamazepine	tba	Every 6 months	Loos et al., 2013
Difficult to degrade biologically; not amendable to chemical oxidation[3]			
Tris (2-carboxyethyl)phosphine (TCEP)	tba	Every 6 months	Loos et al., 2013
Sucralose	tba	Every 6 months	Loos et al., 2013

[1] Biodegradable during conventional activated sludge treatment or biofiltration.

[2] Not degradable during conventional activated sludge treatment or biofiltration, but amendable to chemical oxidation.

[3] Not degradable during conventional activated sludge treatment or biofiltration, not amendable to chemical oxidation.

[4] One of the measurements to be carried out in the summer period.

[5] PNEC – Predicted No-effect Concentration

tba - to be added

An exceedance of any of the threshold values (measured environmental concentration, MEC, above ELV) at the point of compliance (i.e. in the effluent before the mixing zone) and the recommended associated follow-up action is described in the following section.

This guidance on thresholds for each of these tiers is based on conservative values because of the limited toxicological information available and the fact that the suggested point of compliance does not represent the point of exposure of river fauna and flora to the pollution.

It is recommended that the WWTP operator confers with the local environmental regulator to develop a response plan with specific actions to be implemented by the WWTP as part of interpreting appropriate responses to the monitoring results.

One important difference of this approach compared to the one described in Annex 1 is that here only one analytical measurement of the indicator substances in the effluent of the WWTP is required. The approach proposed in Annex 1 requires, instead, two measurements, i.e. one in the influent and one in the effluent of the WWTP.

Table 2 - Proposed actions in case of exceedance of any of the threshold values (measured environmental concentration, MEC, above ELV)

Status	Recommended action
If 1 < MEC/ELV < 10	Quality check data, continue to monitor every three months, until 1 year and until the MEC/ELV < 1 and preferably is consistently less than 5 times the ratio of MEC/ELV
10 < MEC /ELV < 100	Data check, immediate re-sampling and analysis to confirm MEC. Continue to monitor every three months, until 1 year and until the MEC/ELV<1 and preferably is consistently less than 5 times the ratio of MEC/ELV
10 < MEC /ELV < 100	Data check, immediate re-sampling and analysis to confirm MEC. Continue to monitor every three months, until 1 year and the MEC/ELV<1 and preferably is consistently less than 5 times the ratio of MEC/ELV
100 < MEC/ELV < 1,000	All of the above plus enhance source identification programme. Also monitoring in the distribution system closer to the point of exposure to confirm attenuation of CEC is occurring and to confirm the magnitude of assumed safety factors associated with removal efficiency, dilution and post-treatment
MEC/ELV > 1,000	All of the above plus immediately confer with the local environmental authority to determine the required response action. Confirm plant corrective actions through additional monitoring that indicates the CEC levels are below at least a MEC/ELV of 100

An additional list of ecology-related RBSPs addressing regional or local conditions should be defined by the Member States at national or river basin level.

The limit values for these RBSPs should be related to the associated EQS values using an appropriate dilution factor used at the definition of 'mixing zones' according to the EQS Directive (2008/105/EC).

In case of exceedance of ELVs, WWTP operators should confer with the local environmental regulator to develop a response plan with specific actions (same as those identified in Table 2 above).

As regards the bioassays, assessment of risk of mixtures of pollutants in WWTP effluents released into the receiving water body, a list of endpoints (and proposed bioassays) and actions to be taken by WWTP operators in case of exceedance of Effect-based Trigger Values (EBTs) is reported in Table 3.

Table 3 - Effect-based trigger values (EBTs) for a battery of *in vitro* bioassays(*) indicating potential ecological risk

Activity	EBT[a]	EBT[b]
Estrogenic (ERα)[c]	0.5 ng E2-eq/L	**0.1** ng E2-eq/L
Anti-androgenic (anti-AR)	25 µg Flu-eq/L	**14** µg Flu-eq/L
Glucocorticoid (GR)	**100** ng DEX-eq/L	-
Dioxin-like (DR)	**50** pg TD-eq/L	50 pg TD-eq/L
PPARγ receptor (PPARγ)	**10** ng Ros-eq/L	-
Toxic PAHs (PAH)	150 ng BaP-eq/L	**6.2** ng BaP-eq/L
Oxidative stress (Nrf2)	**10** µg Cur-eq/L	21 µg Cur-eq/L
Pregnane X receptor (PXR)	**3** µg Nic-eq/L	54 µg Nic-eq/L

(*) The bioassays referred to in this table are all CALUX cell lines and the reported EBTs are specific to these cell models. For some of the endpoints, there is some variability depending on the cell model used. EBTs for other cell lines can be found in Escher at al., 2018, Sci. Tot. Environ., 102, 343-358.

a Expressed as equivalents of the reference compounds E2 = 17β-estradiol; eq = equivalent; Flu = flutamide; DEX = dexamethasone; T = 2378-TCDD; Ros = rosiglitazone; BaP = benzo[a]pyrene; Cur = curcumine; Nic = nicardipine; van der Oost et al., 2017.

b As above, Escher at al., 2018, Sci. Tot. Environ., 102, 343-358.

The sample location and frequency for these bioassays should be linked to specified monitoring requirements for CECs (i.e., frequency of six months; collected at the point of compliance, which is in this case urban wastewater treatment plant (UWWTP) effluent).

Due to the limited sensitivity of the bioassays it is recommended to enrich the water via solid-phase extraction prior to testing using appropriate solid phase materials.

A proposal discussed during the NEREUS Action would be that the lowest (cf. Table 3, EBTs in bold letters) EBT is taken for the comparison with the signal obtained by any of the used bioassays with the same Mode of Action (Table 3).

The sample location and frequency (proposed once in six months) for these bioassays should be linked to specified monitoring requirements in the WWTPs. An exceedance of the above-proposed trigger values should trigger appropriate specific actions (see Table 4).

Table 4 - Proposed actions in case of exceedance of EBTs

Status	Recommended action
Measured value/EBT < 1	No further action required
1 < measured value/EBT < 3	Quality check data, continue to monitor every three months, until 1 year and until the EBT < 1
3 < measured value/EBT < 10	Data check, immediate re-sampling and quantify specific target compounds which are known to cause the effects observed in the respective bioassay (toxicity drivers). Continue to monitor every three months, until 1 year and the EBT < 1
10 < measured value/EBT < 100	All of the above plus enhance source identification programme. Also monitoring of influent wastewater to confirm the magnitude of assumed safety factors associated with removal efficiency by the available WWT technology and dilution in the receiving water body.
Measured value/EBT > 100	All of the above plus immediately confer with the local environmental authority to determine the required response action. Confirm WWTP corrective actions through additional monitoring that indicates the measured value/EBT ratio is below at least 100

This proposal is based on recent publications by van der Oost et al. (2017a) and Escher et al. (2018) where a selection was made of market-ready relevant and cost-effective bioanalytical endpoints to cover a wide spectrum of CECs' modes of action. Specific endpoints may indicate which classes of chemicals might cause adverse effects. EBTs were derived for these bioassays to indicate potential ecotoxicological risks (Table 5). Comparison of EBTs with bioassay responses should discriminate sites exhibiting different chemical hazards. This would mean that proposed ranges of EBTs for the here discussed bioassays and Modes of Actions would be as in Table 5.

Table 5 - List of applied bioassays, with their mode of action, reference compounds, proposed effect-based trigger values (EBTs) and ranges of exceedance of EBTs indicating a need for different response actions by WWTP operators (labelled with different font/colour styles; for more details see text).

Mode of Action	Reference compound	Cell TA assay e.g.	EBT	1 to 3-times EBT level *(italic)*	3- to 10- times EBT level (underlined)	10- to 100-times EBT level **(bold)**	Above 100-times EBT level ***(italic bold underlined)***
Estrogenicity (ER)[a]	ng eq E2/l	Human or Yeast	0.1[d]	*0.1-0.3*	0.3-1.0	**1.0-10**	***>10***
Inhibition Androgenicity(anti-AR)[b]	µg eq Flutamide/l	Human or Yeast	14[e]	*14-42*	42-140	**140-1400**	***>1400***
Glucocorticoid receptor activation (GR)	ng eq Dexamethasone/l	Human	100[e]	*100-300*	300-1000	**1000 -10000**	***<10000***
Activation of peroxisome proliferator-activated receptor (PPAR)	ng eq Roziglitazone/l	Human	10[d]	*10-30*	30-100	**100-1000**	***>1000***
AhR receptor activation (PAH)[c]	ng eq B(a)P/l	Rat	6.2[e]	*6.2-18.6*	18.6-62	**62-620**	***>620***
Adaptive Stress (Nrf2)	µg eq Dichlorvos/l	Human	10[d]	*10-30*	30-100	**100-1000**	***>1000***
Activation pregnane x receptor (PXR)	µg eq Nic-eq/l	Human	3	*3-9*	9-30	**30-300**	***>300***

[a]: Estrogenicity testing according to ISO19040 and OECD TG455.
[b]: Anti-androgenicity testing according to OECD TG458.
[c]: AhR receptor activation testing according to ISO standards (in progress).
[d]; Escher at al., 2018, Sci. Tot. Environ., 102, 343-358.
[e]; Van der Oost et al., 2017, Environ. Toxicol. Chem 36, 2385-2399.

A model based on similar principles as the one described above was designed to estimate the overall risks for aquatic ecosystems. The associated follow-up for risk management was proposed as a 'Toxicity Traffic Light' (TTL) system. Its potential to become the first bioanalytical tool to be applied in regular water quality monitoring programmes has been successfully tested in the Netherlands (van der Oost, 2017b). The bioassays screening programme as described in Table 5 was successfully tested on effluents from 12 WWTPs in nine countries in a study organised by the International Commission for the Protection of the Danube River in 2017 [11].